Sexual size and shape difference in the crested newt (*Triturus carnifex*): ontogenetic growth aspects

D. Cvetković *, M. L. Kalezić * & G. Džukić **

* Institute of Zoology, Faculty of Biology, Studentski trg 16, 11000 Beograd, Yugoslavia
** Institute for Biological Research "Siniša Stanković", 29 Novembra 142, 11000 Beograd, Yugoslavia

The patterns of emergence and development of sexual size and shape differences (SSSD) in the crested newt were examined. The results of our study indicate that differences in size and shape between females and males appear mostly in the period between the first and the second hibernation, which seems particularly important for development of SSSD. Concerning the questions of time and speed of morphological divergence between sexes, we can conclude that the establishment of SSSD precedes reproduction. The process is not gradual; two periods can be distinguished. The second period is characterized by rapid change, resulting in considerable intersexual differences in various parameters. Juveniles exhibited pronounced sexual dimorphism of growth rates, as well as differences in level and timing of departures from isometric growth, and in values of the Wolterstorff index (WI). The existence of two distinct periods in development of SSSD was also confirmed at the multivariate level.

INTRODUCTION

European newts (*Triturus*, Salamandridae) have the most pronounced morphological sexual dimorphism among tailed amphibians, especially during the breeding season. Sexes differ in coloration, skin texture and glandular development, as well as in body size and shape. In most of 12 extant species, including *Triturus carnifex*, females are larger than males (Kalezić et al., 1992). Significant differences were found for most linear dimensions (measures of size), as well as for some ratios of these dimensions (measures of shape), though shape differences have attracted less interest so far. It was established that the extent of sexual size difference in European newts is a variable condition not constrained by species body size, spatial proximity of analysed populations or altitude (Kalezić et al., 1992).

However, patterns of emergence and development of sexual size and shape differences (SSSD) still remain to be clarified. Many questions can be raised concerning the time and speed of morphological divergence between sexes. Do the differences in body size and shape fully develop prior to attainment of sexual maturity? Is the process of divergence

gradual during the juvenile phase of life or can a distinct period of rapid change be observed? Moreover, it is still unknown which morphometric characters are most involved in the above process.

Growth before the age of first reproduction (larval and juvenile stages) appears to be the major determinant of body size in amphibians and reptiles in general (HALLIDAY & VERRELL, 1988), and in newts in particular (e.g. HAGSTROM, 1980; GLANDT, 1981; VERRELL & FRANCILLON, 1986; KALEZIĆ et al., 1994).

Thus, as the main concern of this paper was to find the pattern of SSSD appearance in *Triturus carnifex*, we have studied morphometric growth aspects during the critical ontogenetic period between the first and the second hibernation.

MATERIALS AND METHODS

LABORATORY PROCEDURES

Crested newt specimens were collected from Lokanj pond (Montenegro) in October 1991. We sampled 43 juveniles, as well as fully mature individuals, 21 females and 19 males ("adults" in the following text). Newts were anaesthetized by immersion in a 2:1000 MS-222 (Sandoz) solution, individually marked by toe clipping and measured. Shortly after measuring, the newts were put into hibernation in a cold room at 7.5°C till next February. On emergence from "wintering", 43 juveniles (all survived hibernation) were measured again and housed in a 300 l aquarium. This aquarium had been established three years before and contained diverse weeds, simulating a natural pond. It was exposed to a natural photoperiod and daily changes in room temperature (10-25°C); the constant water level was maintained. Water was continually recycled and filtered. Thus, the newts were maintained in standard conditions with ad libitum access to food (earthworms and pieces of beef meat). Juveniles were measured each subsequent 30 days for another 10 months. Body mass was determined by blotting individuals dry and weighing them to the nearest 0.01 g

Out of 43 juveniles, 23 survived till the second hibernation, 15 females and 8 males. They were sexed according to the presence of secondary sexual characteristics, the identification of males being easier through the appearance of a dorsal crest, a thin crenulated skin flap, a bluish-white streak along the tail and a swollen black cloaca. At that age, exclusively female secondary sexual feature, the presence of cloaca papillae, was less apparent.

MORPHOMETRIC VARIABLES

In juveniles and adults, 9 morphometric characters were measured: SVL (snout-vent length), Lcp (the distance from the snout to the frontal edge of the cloaca), Ltc (head width at the angle of the jaw), Lc (head length from the snout to the corner of mouth),

Lcl (distance between the snout and the gular skin fold), Pa (fore limb length, measured from axilla to the tip of the longest finger), Pp (hind limb length, from groin to the tip of the longest finger), D (distance between fore and hind limbs) and Lh (the maximum fin height measured at the base of the tail). All measurements were made with a dial caliper with 0.1 mm precision.

STATISTICAL ANALYSIS

Two data sets are presented here: one consists of records of the same individual at different time points, the other is based on samples of different individuals at the same time point. According to COCK (1966), they are defined as longitudinal and static data, respectively. Consequently, we distinguish growth allometry, changes in size-shape relationships with time, from static allometry, based on records of different individuals (e.g. GOULD, 1966).

The growth of juvenile newts was expressed through relative growth rate (K), calculated according to the following equation (ANDREWS, 1982).

$$K = (lnS_2 - lnS_1) / (t_2 - t_1),$$

where S_1, S_2 and t_1, t_2 were size (standard length) at and time of two measure points. Linear regressions of SVL on time were also calculated for both groups of juveniles

The Wolterstorff index (WI), widely recommended as a useful tool for distinguishing crested newt taxa (e.g. HERRE, 1932; SOVA, 1973, KALEZIĆ et al., 1990; LANZA et al., 1991), was calculated as the ratio of forelimb length to interlimb distance (WOLTERSTORFF, 1923). Apart from various uni- and bivariate statistical analyses (SOKAL & ROHLF, 1981; ZAR, 1984), multivariate procedures of principal-component analysis (PCA) and Mahalanobis distance (D^2) were applied. Due to the small sample size, multivariate procedures were conducted on the set of seven morphometric traits (SVL, Ltc, Lcl, Pa, Pp, D, Lh) in juveniles and adults.

Principal-component analysis allowed simultaneous analysis of morphometric data, reducing dimensionality but retaining variation. It was performed on the variance-covariance matrix of log-transformed data, a procedure recommended when dealing with morphometric traits (e.g. BOOKSTEIN et al., 1985). Separate principal-component analyses were computed for 4 time points (I: prior to the first hibernation; IV: April; VIII: August; XI: November, prior to the second hibernation) in juveniles and for adults. The Mahalanobis multivariate distance between the sexes was computed for all measurement points. Mantel test was used to analyse the correspondence of character variance-covariance matrices.

RESULTS

The range of standard length of 43 juveniles was 44.1-55.9 mm (mean ± standard error: 51.6 ± 0.4 mm). This indicates that they hatched in the same season (spring 1991),

metamorphosed during a relatively short time span and therefore were suitable for this study, though there is evidence of a plastic life-history including facultative paedomorphosis in the Lokanj population (KALEZIĆ et al., 1994). The mean SVL of 23 surviving juveniles at the beginning of the study was 51 7 ± 0.6 mm (range 47.9-55.9 mm) for females and 51.1 ± 0.6 mm (range 48.8-53.7 mm) for males, the difference being statistically insignificant (ANOVA, $F - 0.445$, $P > 0.05$). The mean value of SVL prior to the second hibernation was 75.2 ± 0.8 mm (range 69.7-79.1 mm) and 69.9 ± 1.9 mm (range 56 9-73.8 mm) for females and males, respectively. Intersexual difference was significant (ANOVA, $F = 8.74$, $P < 0.01$).

It was essential for our laboratory study to avoid competition for food among juveniles and therefore allow the undisturbed development of SSSD. When density is low (intraspecific competition low or absent), a normal distribution of individual growth rates is expected. Otherwise, intense competition for food would result in a skewed or lognormal distribution (WILBUR & COLLINS, 1973; WILBUR, 1976). The distribution of growth rates in our data set showed good fit to normal distribution (Kolmogorov-Smirnov test, $P > 0.05$ in all cases). Thus, we can conclude that nutritional conditions were similar for all individuals, i.e. there was no size-dependent advantage.

The analysis of relative growth rates indicated the existence of two distinct periods. Up to July (the first half of the year) females invariably had faster growth, but the difference was insignificant (ANOVA, $P > 0 05$ in all cases). The second period is characterized by significantly increasing differences (ANOVA, July-August, $P - 0.01$; August-September, $P = 0.05$; September-October, $P < 0.01$), except for the last month before the second hibernation ($P > 0.05$). The comparison of linear regressions of SVL on time over the whole time span studied indicated that females were growing significantly faster than males (b♀ = 2.436 ± 0 100, b♂ = 1.925 ± 0.097; t test, $t = 3.66$, $P < 0.001$).

It is of interest to note negative growth during hibernation. Indeed, the comparison of the first two measurement points showed a significant decrease, not only in weight (paired t test, $t = 15.87$, $P < 0.0001$), but in length as well (paired t test, $t = 4.39$, $P < 0.001$), with pronounced individual variability.

In the sample of adults, females were larger than males (mean SVL 74.4 ± 1.4 and 72.6 ± 1.0, respectively); however, the difference was insignificant (ANOVA, $F = 1.16$, $P > 0.05$).

BIVARIATE ALLOMETRY

In order to analyse the pattern of changes in bivariate allometric coefficients during ontogeny, regression analysis was performed on eight morphometric traits (using SVL as the independent variable) for all measurement points in juveniles, as well as for adults (tab. 1). Intersexual difference in departures from isometric growth is obvious in the number of statistically significant allometric coefficients. In juvenile males, out of 88 values, 18 were statistically significant (20.4 %) vs. 6/88 (6.8 %) in juvenile females (χ^2, $P < 0 05$). Also, in males, contrary to females, most of the significant values appear in the second half of the year. Negative allometry is apparent: 5/6 significant coefficients in

Tab. 1. - Significant bivariate allometric coefficients in males and females. Positive allometry:
+, P < 0.05; + +, P < 0.01. Negative allometry: −, P < 0.05; − −, P < 0.01; − −
−, P < 0.001. I - XI: measurement points in juveniles; AD: adults.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	AD
♂												
Lcp			−									
Ltc		+					− −		− −	− −	− −	− −
Lc										−		
Lcl			−							−	− −	
Pa		−				+			+ +			
Pp												
D							+	+				
Lh							+					+
♀												
Lcp				+ +								
Ltc												
Lc												
Lcl		−										−
Pa												
Pp				−							−	
D	−											− − −
Lh												

juvenile females, 2/2 in adult females and 12/18 in juvenile males. Traits showing
considerable ontogenetic allometry are: Lcp, Ltc, Lcl and Pa in males, Pa and Pp in
females.

WOLTERSTORFF INDEX AND MAHALANOBIS DISTANCE

Morphometric differentiation between the sexes in terms of multivariate distance
(Mahalanobis D^2) and bivariate parameter WI (Wolterstorff index) is shown in fig. 1.
Mahalanobis distance changed slightly between measurements I and VII (prior to the
first hibernation and next July) and then increased rapidly. At the time of the
second hibernation it was even higher than the value found in the sample of adults
(D^2_{ad} = 6.02).

Intersexual differences in WI values, characteristic for some salamander species
(particularly crested newts), were also calculated for all measurement points. The pattern
of change followed that of Mahalanobis distance − rapid change in the second half of the
year resulted in highly significant differences between sexes. The value just prior to the
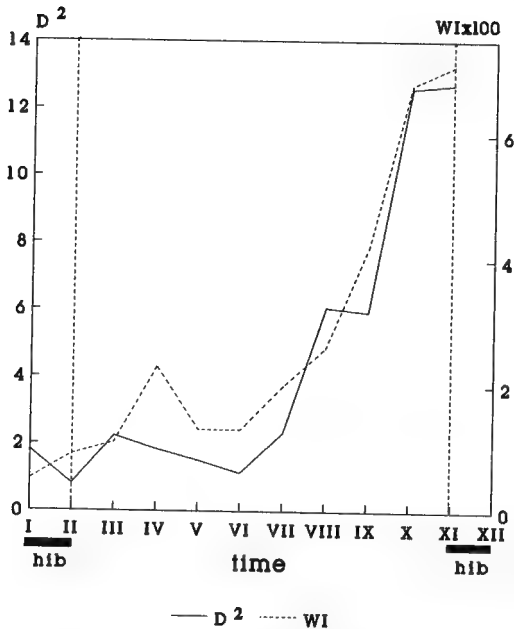second hibernation (0.071) was somewhat higher than in adults (0.062).

Fig 1. – Mahalanobis distance (D^2) and differences in values of Wolterstorff index (WI) between juvenile males and females, hib: hibernation. Measurement points II – XI correspond to months (February to November).

DEVELOPMENT OF SSSD IN MULTIVARIATE SPACE

Mantel test was applied to examine the correspondence of character variance-covariance matrices between the sexes. The absence of significant positive correlations indicated the differences in character covariance structure between females and males, juveniles as well as adults.

The results of PCA showed that the first two principal components account for a considerable amount of total variation: 74.2-95.1 % in juvenile males, 91.0 % in adult males, 78.7-86.2 % in juvenile females and 88.7 % in adult females. This indicates strong correlations between variables, as is expected when dealing with morphometric traits. Principal-component 1 can be considered a general size component, while PC2 represents a shape component. The magnitudes of eigenvector elements indicate the contribution of each original trait to principal components (fig. 2).

Principal-component 1 measures variation attributable to differences in size. All traits load on PC1 positively. Similar, but not identical, values of eigenvector coefficients indicate the influence of allometric information. The most striking feature is the position of fin height (Lh) in PC1-PC2 space. This trait dominates both axes and is opposed to all other traits (this is less pronounced only for measure point IV in females). It has a constant, stable position in morphological space, irrespective of sex, the only exception being adult males. In adult males, the position of sets of traits is reverse, compared to females, due to opposite signs of eigenvector loadings on PC2.

Traits related to locomotion, Pa and Pp, load as a group and show some intersexual differences. Other traits do not show a recognizable pattern. Snout-vent length has a relatively stable position in juveniles, irrespective of sex. Variation in head dimensions, related to feeding (Ltc and Lcl) and in interlimb distance (D) shows no simple trend during ontogeny, though intersexual differences appear in the youngest juveniles (measurement points I and IV).

The average values of PC scores (fig. 3) show time-related changes in size and shape of studied individuals. The last pair of points represents adult males and females occupying different morphological spaces; shape divergence is mostly influenced by one trait, fin height.

THE ATTAINMENT OF SEXUAL MATURITY

The newts with well expressed secondary sexual characteristics were allowed to court and oviposit shortly after the second hibernation. We observed courtship and spermatophore deposition, but no oviposition took place during the expected breeding season.
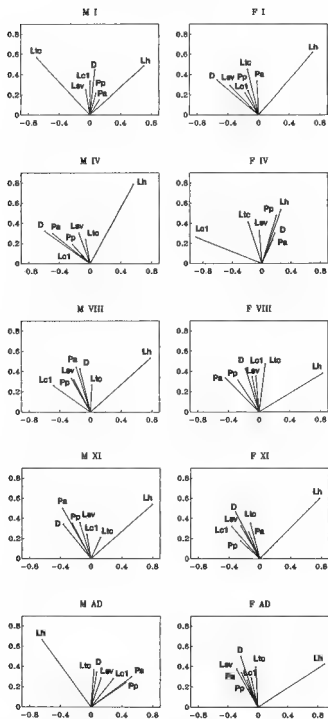
Fig. 2. · Plots of eigenvectors (X axis PC2, Y axis PC1) for 4 measurement points in juveniles (I, IV, VIII, XI) and for adults (AD); M: males, F: females.
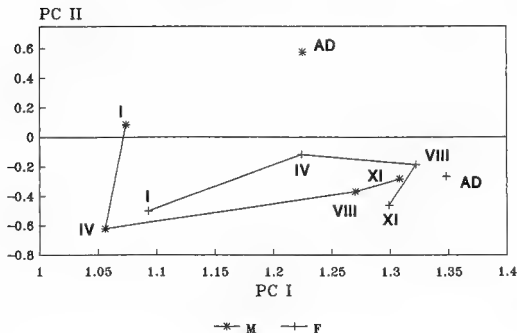
Fig. 3.   Average values of PC scores for 4 measurement points in juveniles (I, IV, VIII, XI) and for adults (AD); M: males, F: females.

DISCUSSION

The results of our study indicate that differences in size and shape between females and males appear mostly in the period between the first and the second hibernation, which seems particularly important for development of SSSD. At least some juvenile crested newts, maintained in our laboratory, attained sexual maturity at the age of 2 years. This seems to be the minimum age at first breeding for *Triturus cristatus* superspecies; in the field it usually takes longer — from 3 to 5 years (HAGSTROM, 1975; FRAZER, 1983; FRANCILLON-VIEILLOT et al., 1990).

Thus, concerning the questions of time and speed of intersexual morphological divergence, we can conclude that the establishment of SSSD precedes reproduction The process is not gradual; a period of rapid change can be distinguished, resulting in considerable intersexual differences, confirmed by various uni-, bi- and multivariate analyses.

In species with intersexual size difference, several patterns of juvenile growth are possible (ANDREWS, 1982). One is for juveniles to grow at similar rates until individuals of the smaller sex reach asymptotic length, while the larger sex continues to grow, so the growth curves diverge. Another pattern is to grow at different rates, either from the beginning (hatching) or from some point early in life. In our study, juveniles exhibited a

pronounced sexual difference in growth rates, according to the second pattern. Greater importance of juvenile compared to adult growth rates has been stressed before (e.g. HALLIDAY & VERRELL, 1988).

As far as adults are concerned, in the crested newt populations females are almost invariably larger than males (KALEZIĆ et al., 1992). In this sample of Lokanj adults, the difference is not significant. However, another sample from the same population obtained significant sexual size difference (unpublished results). The variation of body size distributions and the extent of sexual size dimorphism at the inter- and intrapopulation level is a common phenomenon (HALLIDAY & VERRELL, 1988; STAMPS, 1993). Many factors can be responsible for this variation among the samples from the same population, such as differences in growth rates or age structure (STAMPS, 1993).

Our results show a substantial sexual size divergence during the critical period prior to sexual maturation, but the interpretation of adult size data requires more detailed information on adult growth patterns.

Intersexual shape differences were revealed by various bi- and multivariate methods. In terms of bivariate allometry, considerable differences in level and timing of departures from isometric growth are found between females and males. Allometry is mostly negative with respect to standard length. However, allometric coefficients for Pa, the trait involved in WI, show intersexual differences. If we compare all coefficients, values are almost without exception negative in females and positive in males. Hind limb length (Pp) shows mostly isometric (to slightly negative allometric) growth in males and negative allometric growth in females. This confirms some previous findings. REHAK (1983) found for the crested newt females relative shortening of legs with respect to body length. This difference in limb lengths between the sexes might be associated with the courtship behaviour in males and sperm transfer (e.g. HALLIDAY, 1977; REHAK, 1983; RAXWORTHY, 1989).

The existence of two distinct periods in development of SSSD was confirmed at the multivariate level (Mahalanobis distance). Differences in values of the Wolterstorff index exhibited the same pattern, which confirms that this bivariate parameter is a good indicator of size-shape changes. Principal-component analysis showed that the trait with the largest contribution to both components, PC1 and PC2, was fin height (Lh). Sets of traits had a reverse position in adult females and males, occupying different morphological spaces, mainly due to shape differences.

We are aware that the paucity of sample size in our study, especially of males, precludes well-supported conclusions, though general trends are apparent. Additional data are needed for a more rigorous assessment of these trends.

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